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Characterization of Cerebral Blood Oxygenation and Flow Changes During Prolonged Brain Activation

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Abstract: The behavior of cerebral blood flow and oxygenation during prolonged brain activation was studied using magnetic resonance imaging (MRI) sensitized to flow and oxygenation changes, as well as positron emission tomography sensitized to flow. Neuronal habituation effects and hemodynamic changes were evaluated across tasks and cortical regions. Nine types of activation stimuli or tasks, including motor activation, vibrotactile stimulation, and several types of visual stimulation, were used. Both flow and oxygenation were evaluated in separate time course series as well as simultaneously using two different MRI methods. In most cases, the activation-induced increase in flow and oxygenation remained elevated for the entire stimulation duration. These results suggest that both flow rate and oxygenation consumption rate remain constant during the entire time that primary cortical neurons are activated by a task or a stimulus. *Hum. Brain Mapping 5:93–109, 1997.* © 1997 Wiley-Liss, Inc.

Key words: BOLD contrast; fMRI; PET; deoxyhemoglobin; neuronal habituation; human brain mapping; neuronal metabolism; brain function; photic stimulation; visual cortex

INTRODUCTION

It has been demonstrated that magnetic resonance imaging (MRI) can be sensitized, with the appropriate pulse sequence and experimental manipulation, to changes in cerebral blood volume [Belliveau et al., 1991], perfusion [Edelman et al., 1994b,c; Kim, 1995; Kwong, 1995; Kwong et al., 1992a, 1995; Wong and Bandettini, 1996; Wong et al., 1996a] and oxygenation [Bandettini et al., 1992; Blamire et al., 1992; Frahm et al., 1992; Jezzard et al., 1994; Kwong et al., 1992a; Ogawa and Lee, 1990; Ogawa et al., 1990a,b, 1992; Stehling et al., 1993; Turner et al., 1991, 1993]. These sensitizations have allowed MRI to be used for noninvasive mapping of human brain function [Bandettini et al., 1992; Belliveau et al., 1991; Edelman et al., 1994a; Frahm et al., 1993; Kim, 1995; Kwong et al., 1992a]. The array of MRI techniques used for this purpose has been termed functional MRI (fMRI).

As suggested by results of positron emission tomography (PET) [Fox and Raichle, 1986], optical imaging [Grinvald et al., 1991], and near infrared spectroscopy [Villringer and Dirnagle, 1995; Villringer et al., 1993],

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the fraction of paramagnetic deoxyhemoglobin in the vasculature is locally decreased during brain activation. Delivery of oxygenated blood exceeds metabolic need. This decrease in deoxyhemoglobin causes a reduction in susceptibility-induced magnetic field gradients in the vicinity of veins and red blood cells, causing an increase in spin coherence (decrease in 1/T2*), and therefore an increase in signal in T2*-weighted sequences. The source of this signal change is commonly termed blood oxygenation level dependent (BOLD) contrast (Ogawa et al., 1990b). Because of its robustness and ease of implementation with a variety of pulse sequences and systems, BOLD contrast is the most commonly used neuronal mapping contrast in fMRI.

An important goal in fMRI research is characterization of the coupling among neuronal activity, metabolism, hemodynamic changes, and subsequent changes in the MRI signal. The utility of fMRI as a brain mapping tool is directly related to the degree to which the relationship between MRI signal changes and underlying neuronal activation is established. Research on these topics should yield more quantitative information, more accurate localization, and higher temporal resolution, thereby increasing the utility and interpretability of fMRI and other hemodynamic-based brain imaging methods.

Research directed at characterization of the fMRI signal change *dynamics* during activation is an avenue by which a more precise understanding of the relationship between fMRI signal changes and underlying neuronal changes can be achieved. Several aspects of fMRI signal dynamics have been described previously. These include the time to reach a plateau after stimulus onset [Bandettini et al., 1993a; Blamire et al., 1992; DeYoe et al., 1992; Kwong et al., 1992a], the time to return to baseline after stimulus cessation [Bandettini et al., 1993a; Blamire et al., 1992; DeYoe et al., 1992; Kwong et al., 1992a], and the characteristics of a response to a brief "impulse" of activity [Bandettini et al., 1993b, 1995c; Blamire et al., 1992; Savoy et al., 1994, 1995].

Several other aspects of the fMRI signal remain controversial, suggesting processes other than simply large flow changes coincident with minimal metabolic changes. These include the observation by a few groups of the initial undershoot or "dip" occurring 0.5 sec [Ernst and Hennig, 1994; Hennig et al., 1995] to 2 sec [Le and Hu, 1996; Menon et al., 1995a,b] after stimulus onset. Another phenomenon, a postactivation undershoot, is variably observed [Frahm et al., 1992; Kwong et al., 1992a; Ogawa et al., 1992], and the reason for its appearance remains unknown. Another occasional observation is a downward drift in the baseline [Frahm et al., 1992, 1993]. Lastly, the fMRI signal during prolonged steady-state extended duration stimulation has been studied by several groups [Bandettini et al., 1995a,c, 1996; Frahm et al., 1996; Hathout et al., 1994], with widely varying results. This last issue is the focus of this paper.

Hathout et al. [1994], using a BOLD contrast-based FLASH technique, reported an MRI signal decrease, in the visual cortex, from an initially elevated state, to baseline after about 15 min of continuous visual stimulation. Frahm et al. [1996] observed a return of oxygenation-sensitive MR signal to baseline after about 1–2 min of sustained activation. This group also reported an initial increase (and then decrease, after about 4 min) in lactate concentration and sustained blood flow during the entire stimulation duration. This set of observations suggests a transition from anaerobic to aerobic metabolism after 2 min of sustained stimulation and a corresponding initial uncoupling and then recoupling of oxygen delivery and metabolic demand. After cessation of stimulation, an undershoot lasting about 2–3 min was observed. This undershoot is longer than the typical 1 min undershoot that is more commonly observed.

By contrast, preliminary results from our group [Bandettini et al., 1995a,b, Bandettini et al., 1996] have shown a sustained elevation in *both* BOLD contrast-based signal and flow-sensitive signal, suggesting no return to a resting state coupling. These results have recently been corroborated by similar sustained-activation BOLD signal behavior findings using FLASH and echo planar imaging (EPI) by Howseman et al. [1996] and by Kollias et al. [1996].

Controversy regarding the signal change dynamics may exist partially because the BOLD signal depends on several physiological and physical variables that are not easily separated. As described using several types of biophysical models [Bandettini and Wong, 1995; Boxerman et al., 1995; Kennan et al., 1994; Weisskoff et al., 1994; Yablonsky and Haacke, 1994], the activation-induced BOLD signal change is affected by blood volume and blood oxygenation changes, among other factors. Blood oxygenation is strongly affected by the interplay between blood flow and oxygen consumption. It follows that the MRI signal change dynamics will be strongly affected by the relative dynamics of flow changes, blood volume changes, oxygen consumption changes, and subsequent blood oxygenation changes. The relative effects of different stimuli on the dynamics, locations, and magnitudes of these changes and also of these changes on MRI signal have yet to be determined completely. The work in this paper was

directed at the goal of clarifying some of these issues in the context of extended stimulation.

Two types of experimental approaches are available in analyzing extended stimulation effects—those that involve varying the functional contrast sensitization and those that involve varying the stimulus type. In this paper, the contrast sensitization was varied such that flow and oxygenation changes were separately observed, and the stimulus was varied such that hypothetical differences in neuronal habituation and / or metabolic coupling were tested. The paper is organized into two general types of experiments: 1) fMRI parameter modulation; and 2) brain activation modulation.

The first section consists of six parts, which include: 1a) separate sensitizations to oxygenation and flow using gradient echo [Bandettini et al., 1992; Frahm et al., 1992; Karczmar et al., 1994; Kwong et al., 1992b; Ogawa et al., 1992; Turner et al., 1993] and inversion recovery sequences [Kwong et al., 1992a]; 1b) long TR BOLD contrast to completely eliminate flow sensitization; 1c) incrementation of the echo time (TE) to allow simultaneous observation of flow and BOLD effects; 1d) use of a T2*-weighted RF spin-tagging technique called proximal inversion controlled for off resonance effects (PICORE) [Wong et al., 1996b] to observe oxygenation and flow effects simultaneously; and 1e) spiral scanning [Glover and Lee, 1995; Noll, 1995] to compare the extended stimulation effects of non-EPI sequences.

The second section consists of two parts, which include: 2a) the use of different types of visual stimuli known to activate regions in the visual cortex selectively with different concentrations of mitochondria (“blobs” and “interblobs”) [Silverman et al., 1989]; and 2b) the modulation of brain activation stimuli that correspond to different neuronal habituation effects (i.e., decreased rate of neuronal firing over time). BOLD contrast was used.

Overall, we were able to observe extended duration flow effects apart from extended duration oxygenation effects and were also able to characterize neuronal habituation effects apart from effects due to increases in oxidative metabolic rate.

MATERIALS AND METHODS

Functional MRI using EPI was performed using either a 1.5 Tesla GE Signa scanner fitted either with an ANMR resonant gradient system or with an insertable balanced-torque three-axis local head gradient coil [Wong et al., 1992]. Multi-shot spiral scanning [Glover and Lee, 1995; Noll, 1995; Noll et al., 1995] was also performed in one study. A single plane was obtained, and in-plane motion correction was performed. Studies that demonstrated excessive motion were not included in the results.

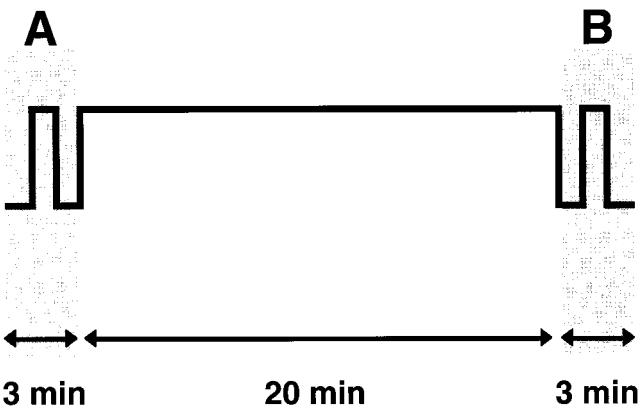

Figure 1.
Schematic diagram of the stimulation timing used in Parts 1a, 1b, and 1d. The timing was: 1 min off, 1 min on, 1 min off, 20 min on, 1 min off, 1 min on, 1 min off. The before and after long-duration activation periods (epochs A and B) were used as control stimuli for assessment of gross motion or drift. The regions of interest used for time course analysis were chosen from voxels that had a response exceeding a correlation coefficient of 0.5 with a box car function for both epochs A and B.

Part 1: MRI parameter modulation

Part 1a: selective oxygenation and flow sensitization

These studies established a preliminary assessment of the behavior of flow and oxygenation in the visual cortex during extended-duration stimulation periods. Flow-sensitive (inversion recovery EPI: TI = 1,000 ms, TR = 3,000 ms, TE = 20 ms; spin-echo) and blood oxygenation-sensitive (gradient echo EPI: TR = 3,000 ms, TE = 40 ms) images were obtained. The voxel volume was 3.1 × 3.1 × 10–15 mm. The stimulus was 10 Hz full-field black and white alternating checkerboard visual stimulation. Timing was: 1 min off, 1 min on, 1 min off, 20 min on, 1 min off, 1 min on, 1 min off. Plots of signal vs. time were obtained from regions of interest created by voxels that demonstrated a correlation coefficient above 0.5 to a boxcar function for both the initial and final off-on-off periods, shown as time periods A and B in Figure 1.

Part 1b: selective oxygenation sensitization

A time course series of blood oxygenation-sensitive images (gradient echo EPI: TE = 40 ms) with an even

longer TR (TR = 10 sec) was used. Voxel volume was 3.1 × 3.1 × 10 mm. The stimulus was 10 Hz full-field black and white alternating checkerboard. Timing was: 1 min off, 1 min on, 1 min off, 20 min on, 1 min off, 1 min on, 1 min off. (again illustrated in Fig. 1). A plot of signal vs. time was obtained in the same manner as in Part 1a. The extended stimulation effects were further characterized by calculating the slope of a linear fit to the MR signal in each voxel in the active region during the entire 20 min stimulation period. From the calculated slopes of these linear fits, the percent signal decrease after 20 min, relative to the initial increase, was extrapolated.

Part 1c: simultaneous flow and oxygenation sensitization I

A time course series of computed R2* and TE = 0 (or S_o) maps were obtained by cyclically incrementing the TE by 5 ms. Sequential echo planar images, having TE values from 30 to 75 ms, were obtained. R2* maps were calculated by a monoexponential fit to the decay curve in each voxel, and S_o maps were calculated by extrapolation of the fitted curves to TE = 0 [Bandettini et al., 1993c; Menon et al., 1993; Glover et al., 1996]. One map was obtained every 10 sec. A sensory-motor cortex activation paradigm (sequential bilateral finger tapping of fingers to thumb at a constant rate) was used. The timing was: 1 min off, 1 min on, 1 min off, 4 min on, 1 min off, 4 min on, 1 min off, 1 min on, 1 min off. Shorter stimulation periods were used in this and some of the following sections, because the motive was simply to compare the results with those of Frahm et al. [1996], which demonstrated a significant signal decrease after only 2 min of stimulation.

Part 1d: simultaneous flow and oxygenation sensitization II

Similar stimulis and timings were used as in Part 1a. The PICORE pulse sequence was used [Wong and Bandettini, 1996]. PICORE is a pulsed arterial spin labeling-based perfusion imaging technique that is very similar to EPISTAR (Edelman et al., 1994b,c] in that arterial blood is tagged by inversion in a slab proximal to the imaging slice. The only difference is that in EPISTAR, the control condition uses a slab-selective inversion pulse distal to the imaging plane to control for magnetization transfer (MT) effects, while in PICORE the control condition uses a non-slice-selective off resonance pulse to control for MT effects without perturbing the magnetization of free water protons anywhere in the subject. The difference between PICORE and flow-sensitive alternating inversion recovery (FAIR) [Kim, 1995; Kwong et al., 1995] is that with PICORE, the tagging inversion pulse is of a slab on one side of the imaging place, while with FAIR, the tagging inversion pulse covers two slabs on both sides of the imaging plane. Lastly, the control condition with FAIR is the inversion of the plane being imaged.

Parameters used for arterial tagging were an inversion slab of 10 cm thickness 1 cm removed from the imaging slice, inversion using a long (30 ms) hyperbolic secant pulse, and an in-plane presaturation pulse to destroy magnetization in the slice prior to application of the inversion tag, to minimize interactions between the tag pulse and the magnetization in the slice (TI = 1,600 ms, TR = 2 sec, TE (gradient echo) = 25 ms). Image voxel volume was 3.8 × 3.8 × 10 mm. This method allowed simultaneous measurement of oxygenation and flow effects. Pairwise subtraction of sequential images canceled out the constant BOLD sensitization. Pairwise addition of sequential images canceled the opposite sign flow sensitization while maintaining constant BOLD sensitization.

Part 1e: multishot spiral scan vs. EPI comparison

To begin determining if some of the discrepancies with other findings [Frahm et al., 1996; Hathout et al., 1994] might be based on differences in MRI scanning techniques, this study used a multi-shot spiral imaging instead of EPI. The pulse sequence parameters (TR/TE/θ = 500 ms/30 ms/30°) were chosen to reduce inflow effects. Images having a resolution of 1.5 × 1.5 × 10 mm were obtained with ten interleaves. Instead of using standard multi-shot FLASH techniques for the comparison, spiral scanning was performed due to its high stability and greater insensitivity to pulsatile motion effects [Glover and Lee, 1995; Noll, 1995; Noll et al., 1995]. The stimulus was a full-field 10 Hz alternating checkerboard for 6 min durations separated by 2 min of darkness.

Part 2: neuronal and physiologic parameter modulation

Part 2a: selective "blob" vs. "interblob" analysis

Specific tissues are known to have different concentrations of cytochrome oxidase, indicating differences in mitochondrial concentration and therefore oxidative metabolic rate [Livingstone and Hubel, 1984]. The effects of these differences on the long-term behavior of the BOLD signal were tested by selective long-term stimulation of "blobs" (regions of high mitochondria

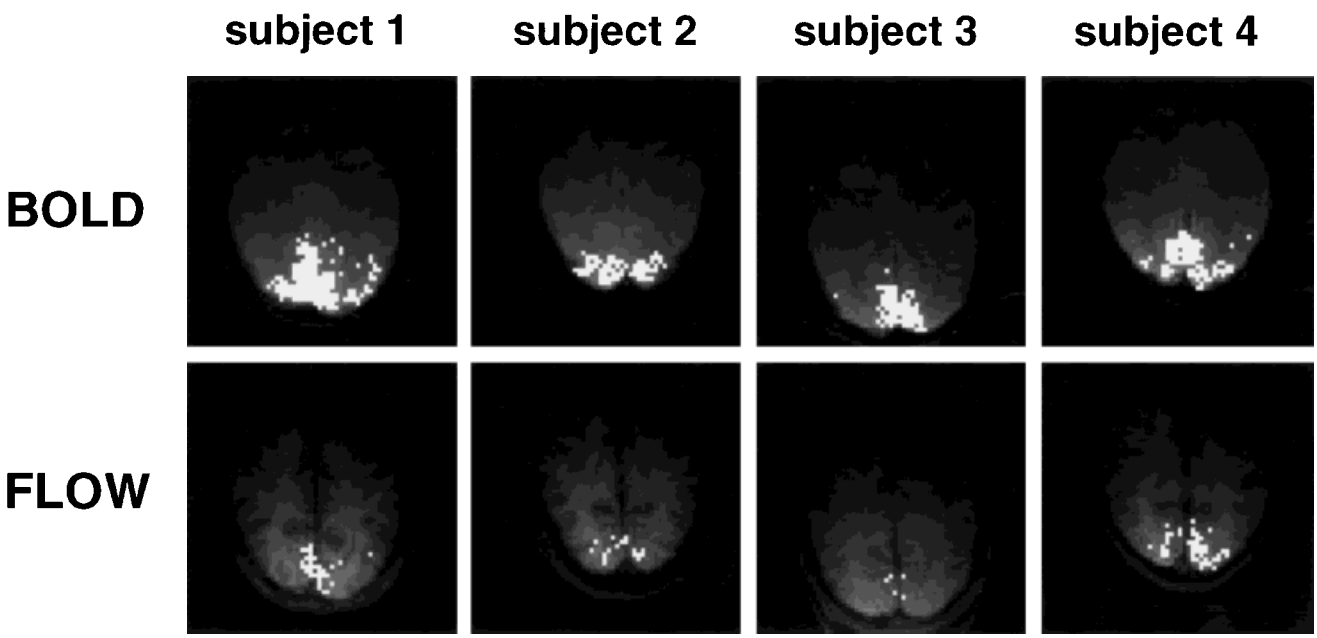

Figure 2.

Activation images obtained from the four subjects in the study in part one. BOLD contrast was achieved using T2*-weighted EPI (TR = 3,000 ms, TE = 40 ms), and flow contrast was achieved using inversion recovery EPI (TI = 1,000 ms, TR = 3,000 ms, TE = 20 ms; spin-echo). Voxels demonstrating a correlation coefficient greater than 0.5 with a box car function for both epochs A and B are shown in white. The signals from these voxels were averaged to create the time course series shown in Figure 3.

concentration) vs. ''interblobs'' (lower mitochondria concentration). The stimuli used to activate blobs were low spatial frequency color stimuli [Silverman et al., 1989]. The stimuli used for activating interblob regions were high spatial frequency black and white stimuli. BOLD contrast was used (gradient-echo EPI, TE = 40 ms). The timing of the blob-only stimulation was 2 min off, 8 min on, 2 min off, 8 min on, 2 min off. The stimulation timing for the direct blob vs. interblob comparison was: 2 min off, 6 min blob stimulation, 2 min off, 6 min interblob stimulation, 2 min off, 6 min blob stimulation, 2 min off, 6 min interblob stimulation, 2 min off.

Part 2b: habituation effect analysis

Toward the goal of characterizing neuronal habituation effects (i.e., decreased neuronal firing over time) on blood flow and oxygenation, stimuli thought to cause different degrees of neuronal habituation (reduction in neuronal firing) were used. BOLD contrast fMRI (gradient-echo EPI, TE = 40 ms) and PET scanning were used. The stimuli used included flashing diffuse red, flashing red LED goggles (Grass), flashing white, alternating checkerboard, continuous ''on'' featureless white stimuli, somatosensory stimuli, and motor activation. The stimuli durations ranged from 6 to 50 min.

With PET, cerebral blood flow was measured 40 sec after bolus intravenous injections of ^{15}O-labeled water at 10 min intervals during 50 min of either continuous 8 Hz full-field alternating red and black checkerboard stimulation or vibrotactile stimulation.

RESULTS

Part 1: MRI parameter modulation

Part 1a: selective oxygenation and flow sensitization

The voxels in the four subjects that demonstrated a correlation coefficient greater than 0.5 for both the first and last 3 min epochs (1 min ''off,'' 1 min ''on,'' 1 min ''off'') are shown in Figure 2. The signals from these voxels were averaged over space, normalized, and then averaged across the four subjects to create the flow-weighted time course series and the oxygenation-weighted time course series shown in Figure 3. For each subject, flow and oxygenation remained elevated for at least the first 10 minutes of stimulation. A small and variable decrease in both flow and BOLD signal occurred in two subjects after about 7 to 10 minutes. An undershoot was observed following all stimulation periods for BOLD

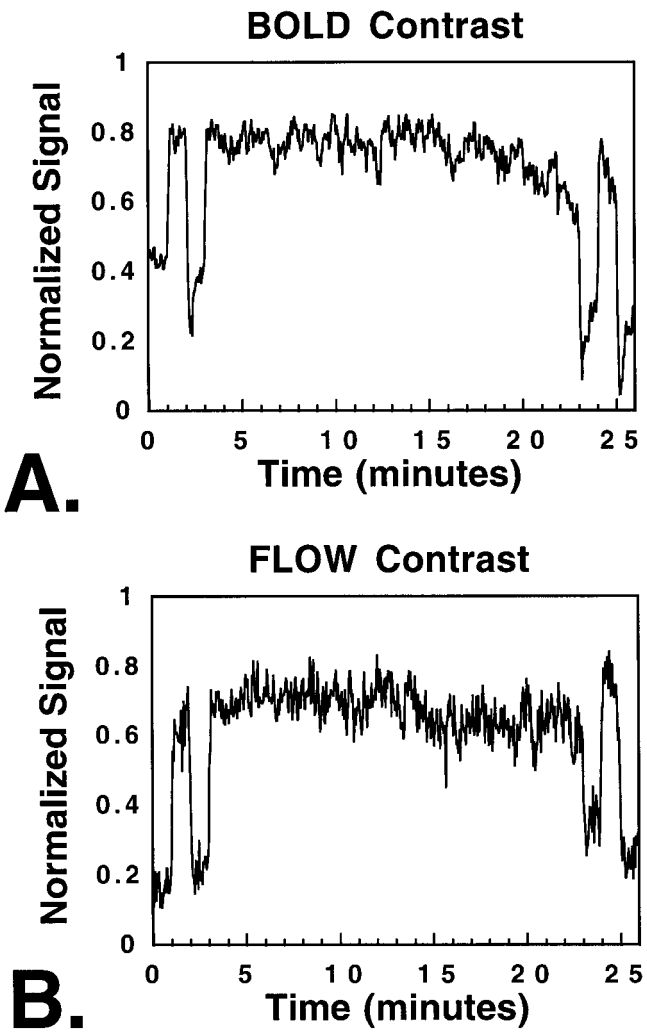

Figure 3.
Averaged (across four subjects) BOLD contrast signal (**A**) and flow contrast signal (**B**) from the visual cortex during extended duration stimulation. Both blood oxygenation and flow remain elevated during the entire 20 min stimulation duration.

weighted sequences but not for flow weighted sequences, as first observed by Kwong et al. [1992a].

Part 1b: selective oxygenation sensitization

Figure 4 is a plot from the voxels that were chosen in the same manner as in Part 1. The BOLD signal, now having contrast completely independent of flow effects, due to the use of a TR of 10 sec, remained elevated for the entirety of the stimulation duration. A simple display of a spatially averaged time course is potentially misleading if significant spatial heterogeneity in the signal response behavior exists. Figure 5 addresses this issue. Figure 5A is an anatomical echo planar image from the time series. Figure 5B shows initial fractional signal changes during activation (average of first 2 min of activation relative to the 1 min period immediately preceding it). The average fractional signal change, relative to baseline, during this time period was 1.75 ± 0.11%. The maximum fractional signal change was 4.9%. Figure 5C shows the calculated percent change (relative to Fig. 5A, the initial fractional signal change) after 20 min. The use of a voxel-wise linear regression calculation and subsequent fractional signal decrease calculation allowed the entire time course to be used in the analysis. The average signal change after 20 min was −0.5% ± 0.46%. The changes ranged from +0.78% to −3.89%.

Part 1c: simultaneous flow and oxygenation sensitization I

The MR signal intensity from the motor cortex during the periodic acquisition of TE-incremented images is shown in Figure 6. Horizontal bars indicate each 4 min epoch during which finger tapping was performed. The relationship between the MR signal intensity and the echo time, TE, and the tissue transverse relaxation rate, R2* (= 1/T2*) is: $S = S_o (e^{-TE \times R2^*})$, where S is the signal at each time point.

From each of the decay curves, values of R2* and S_o were calculated. Maps of activation-induced ΔR2* and ΔS_o were calculated by subtracting all R2* and S_o

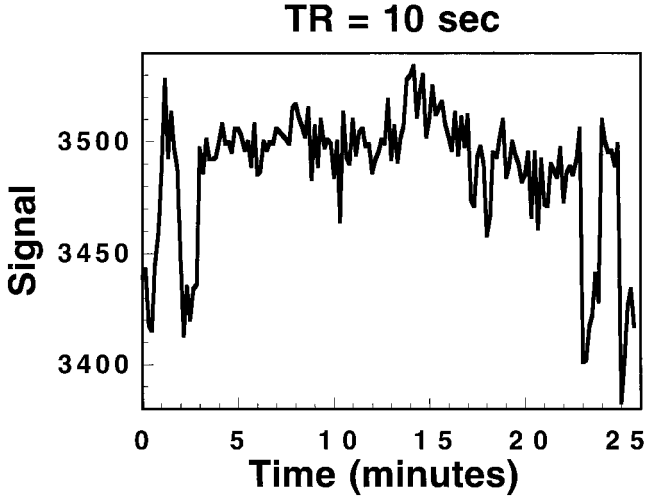

Figure 4.
Time course series obtained using a similarly T2*-weighted sequence as in Part 1a, but with a TR of 10 sec to reduce inflow sensitivity further. The signal, exclusively sensitive to oxygenation changes, remains elevated for the entire 20 min 10 Hz alternating checkerboard stimulation duration.

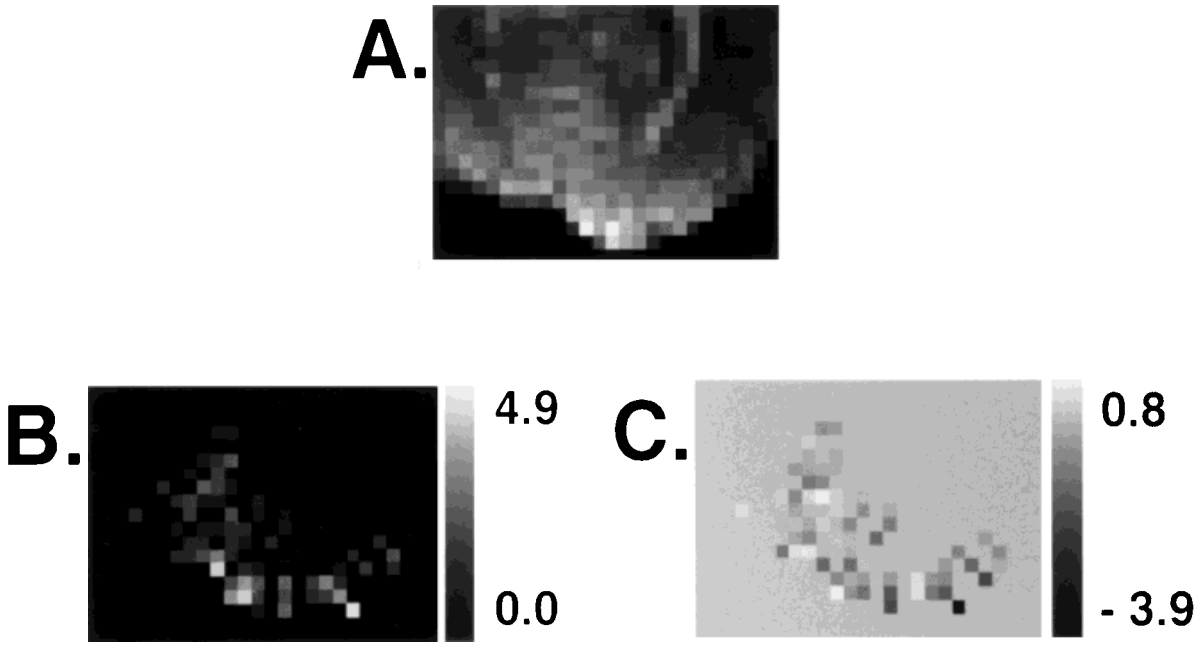

Figure 5.

Assessment of spatial heterogeneity of the extended duration signal changes. Refer to Figure 1 for reference to the stimulation time course. **A:** Anatomical image. **B:** Percent change image: first 2 min of the extended duration stimulation relative to the 1 min baseline (rest state) preceding it. The average fractional signal change, relative to baseline, during this time period was 1.75 ± 0.11%. The maximum percent change was 4.9%. **C:** Calculated percent change, relative to the first 2 min of activation, at the end of 20 min of stimulation. The calculated percent change, after 20 min, relative to the first 2 min, ranged from 0.8 to −3.9%. The average change was −0.5 ± 0.46%. To calculate the maps, first a voxel-wise linear fit to the MRI signal during the 20 min stimulation period was performed. Second, based on the slope of the fitted line in each voxel, the fractional signal change at the end of 20 min was calculated. This allowed the use of the entire time series, rather than only a few images at the beginning and the end of stimulation, to map long-term effects spatially.

images obtained during the ''off'' state from those obtained during the ''on'' state. These maps are shown in Figure 7. The change in S_o was extremely small except for a specific region (appearing bright in the ΔS_o image in Fig. 7). Voxels showing the most robust changes were spatially averaged to create the time course series plots in Figure 8. The time course series from these plots show that R2*, sensitive only to BOLD effects, remained decreased during the entirety of each of the 4 min stimulation durations. Also S_o (independent of BOLD effects and moderately sensitive to inflow effects) remained elevated for the entirety of both stimulation durations.

Part 1d: simultaneous flow and oxygenation sensitization II

Simultaneously obtained flow and BOLD contrast time series, obtained using PICORE and temporally smoothed, demonstrated sustained flow and oxygenation until about 7–10 min and then showed a decrease of about 20% over the remainder of the time course series. The summary of the study is shown in Figure 9. As mentioned, this type of signal decrease behavior was occasionally observed in individual subjects in the above studies.

Part 1e: multi-shot spiral scan vs. EPI comparison

Figure 10 shows a time course MRI signal obtained from the visual cortex obtained using inflow-insensitive spiral scanning (TR = 500 ms, TE = 30 ms, and $\theta = 30°$). The signal remains elevated during the entire 6 min stimulus duration. A post-stimulus undershoot, lasting approximately 1 min, was also observed.

Part 2: physiologic parameter modulation

Part 2a: selective ''blob'' vs ''interblob'' analysis

Figure 11 shows the time course signal from the visual cortex during ''blob'' stimulation lasting 8 min. Two on-off cycles were averaged over time to create

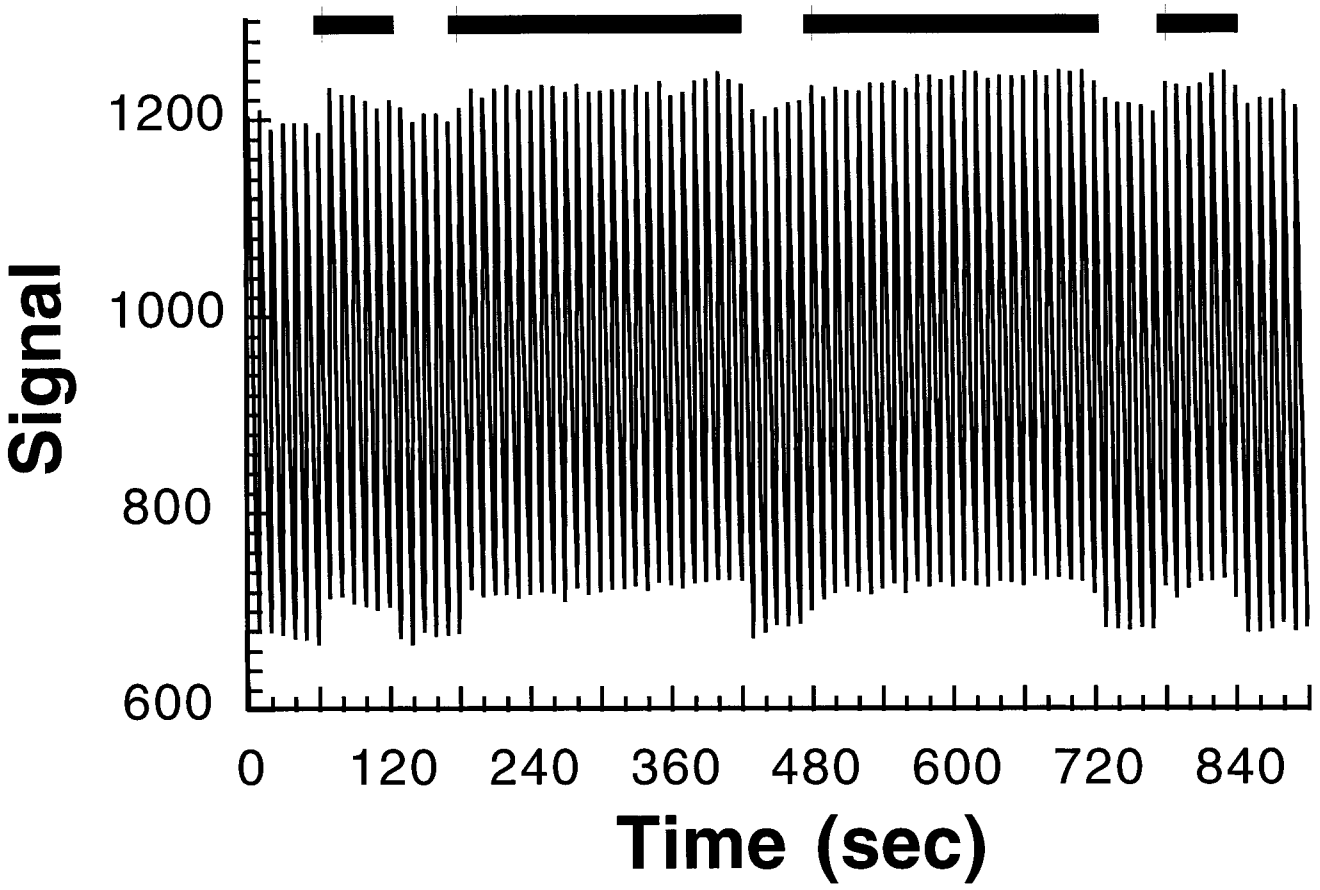

Figure 6.

Signal intensity vs. time from the left and right motor cortex during the collection of echo planar images in which TE was cyclically incremented by 5 ms from 35 ms to 75 ms. From each decay curve a R2* (oxygenation sensitive) and S_o (TE = 0 signal: oxygenation insensitive) was obtained. Horizontal bars indicate the periods when bilateral finger tapping was performed.

the time series display. The BOLD signal remained elevated and did not show a clear undershoot following stimulation. Figure 12 shows a time course comparison in which a diffuse red stimulation (''blob'' stimulation) was alternated with a high spatial frequency black and white alternating pinwheel stimulation (''interblob'' stimulation). The 2 min control periods consisted of darkness. Two on-off cycles were averaged over time to create the time series display. The BOLD signal remained elevated for the entire 6 min blob and interblob stimulation durations.

Lastly, it is interesting to note that, while blob and interblob stimuli gave similar BOLD signal increases, the post-stimulation undershoot was significantly more apparent for the interblob stimulus than for the blob stimulus. Also apparent in Figure 12 is that the interblob stimulus elicited a larger initial signal increase before reaching a steady-state elevated level after about 1 min.

Part 2b: habituation effect analysis

In this section, neuronal habituation effects are demonstrated. Studies using PET are first shown. Figure 13 shows averaged PET measurements across seven subjects for the vibrotactile somatosensory stimulation and across nine subjects for 10 Hz alternating red and black checkerboard visual stimulation. The visual stimulation results demonstrated significantly less decreases in flow, and therefore less neuronal habituation, than did the vibrotactile stimulation.

Figure 14 shows MRI signal intensity from the left and right motor cortex during continuous finger movement for 6 min on each hand. The signal remains

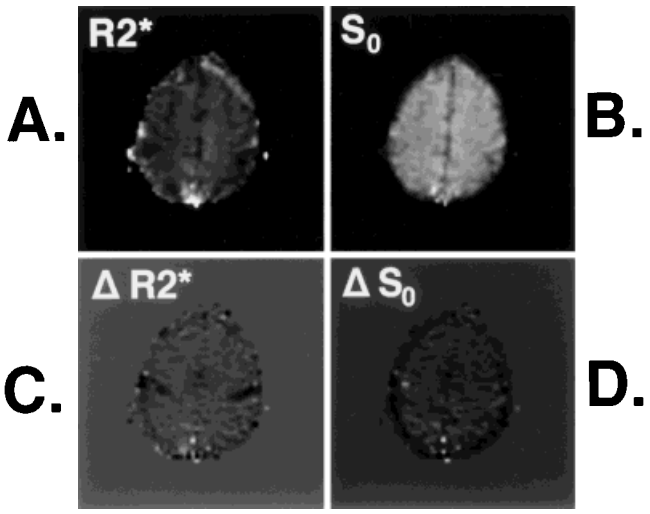

Figure 7.
Resting state maps. R2* map (**A**) and S_o map (**B**) created from the time series of images shown in Figure 6. Activation-induced change maps. ΔR2* map (**C**) and S_o map (**D**) showing oxygenation-related changes (ΔR2*) and inflow-related changes (S_o) with finger tapping. A negative R2* change corresponds to a positive signal change during collection of T2*-weighted images. Minimal inflow changes are generally observed using a TR of 1 sec.

elevated, indicating a lack of neuronal habituation—as would be expected by a motor task as opposed to a somatosensory task. A clear undershoot is also observed after cessation of the finger movement task.

Figure 15 shows the MRI signal intensity from the visual cortex during continuous stimulation with 8 Hz flashing red LED goggles. The stimulation timing and the method by which the region of interest for the time course was chosen were similar to that in Part 1. The signal appears to decrease slowly, after 7 min, to just above half its initial amplitude by 20 min. Given the previous results, it is thought that this signal behavior is due to greater neuronal habituation effects with flashing red stimulation than with alternating black and white stimulation.

A comparison of the effects of a flashing white stimuli with that of a constant (non-flashing) featureless white stimuli, with a baseline of darkness, clearly demonstrates relative neuronal habituation effects. Figure 16 shows the difference in the two effects. Each stimulus was applied for 6 min, with an interval of 2 min of darkness. The flashing white stimulus elicited a sustained response, and the constant (0 Hz) stimulus elicited an initial signal increase followed by a decrease, within 1 min, to baseline. At the transition from darkness to brightness, V1 becomes activated, then returns to baseline after about 1 min if the stimulus does not change.

Figure 17 shows differences across cortical regions in the dynamic effects of the constant featureless white stimuli. On the transition from brightness to darkness, several regions in the anterior (peripheral) V1 show a very brief MRI signal increase that seems to correspond to a brief burst of activity during the transition. This is a clear demonstration of a visual ''off-response,'' with the use of fMRI.

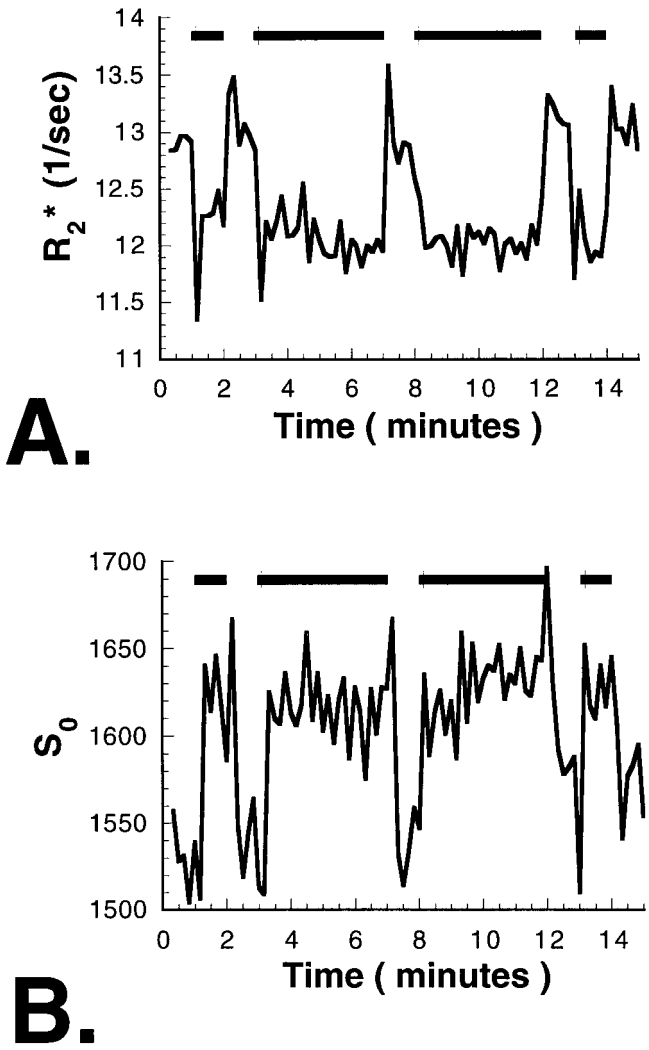

Figure 8.
Time course series of R2* (**A**) and S_o (**B**) changes during extended duration (4 min.) constant rate bilateral finger tapping. The signal was averaged from all voxels showing a correlation coefficient greater than 0.5 with a box car function representing the stimulation timing. The horizontal bars indicate the times during which finger tapping was performed. Both R2* and S_o remain constant during the entire activation durations.

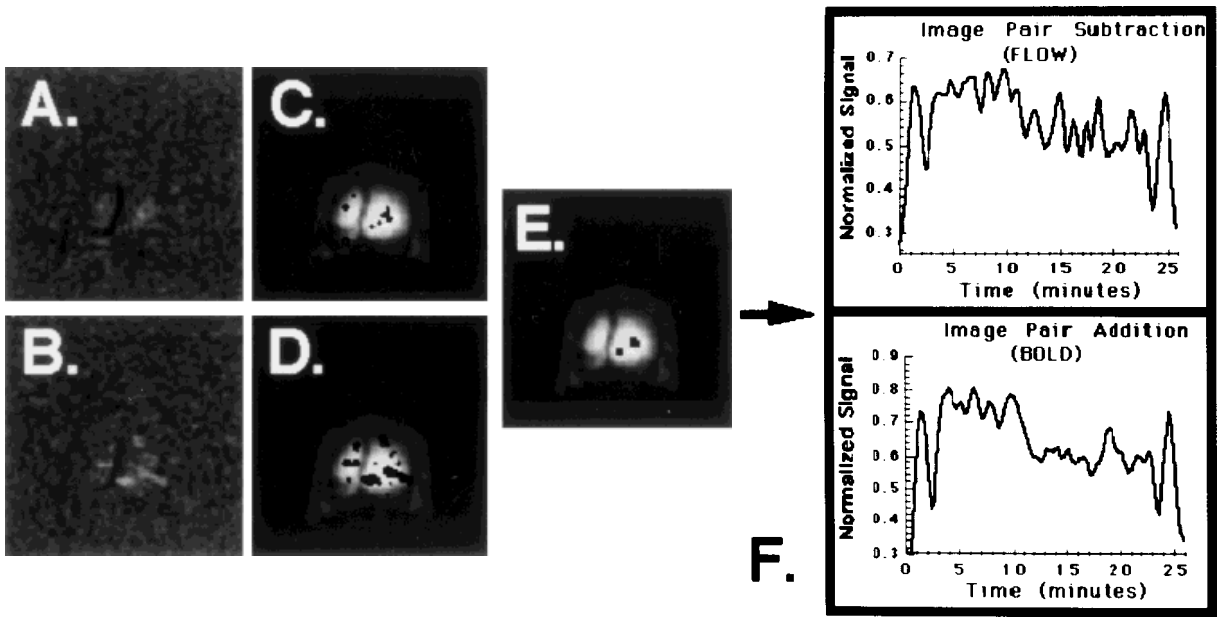

Figure 9.

T2*-weighted PICORE data giving simultaneous flow and BOLD information. The flow-only time course series was created by pairwise image subtraction. The BOLD-only time course series was created by pairwise image addition. **A,B:** Correlation maps for flow and BOLD contrast time series respectively. **C,D:** Functional maps (with threshold applied) for flow and BOLD time series respectively. **E:** Common voxels between C and D from which time course plots were generated. **F:** Temporally smoothed time course data set showing a small signal drop that occasionally occurs in some subjects after about 10–15 min. A small amount of neuronal habituation is hypothesized in this case because *both* BOLD signal *and* flow signal show a decrease at the same time.

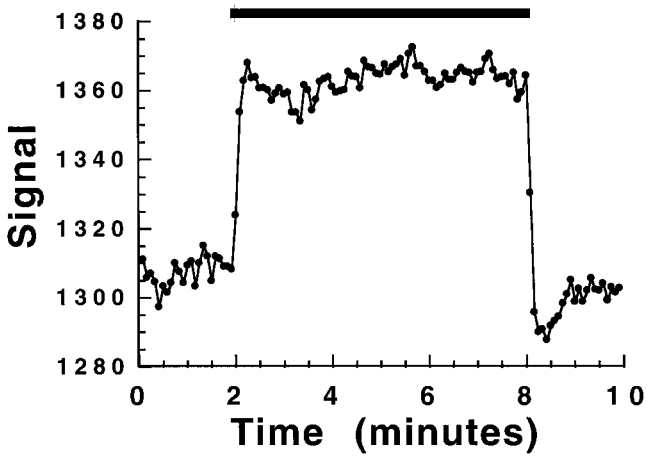

Figure 10.

Blood oxygenation sensitive (TE = 30 ms, TR = 500 ms, θ = 30°) signal from the visual cortex during 10 Hz alternating checkerboard stimulation obtained using multi-shot spiral scanning. The signal remains elevated during the entire stimulation duration and shows an undershoot lasting approximately 1 min following the stimulation.

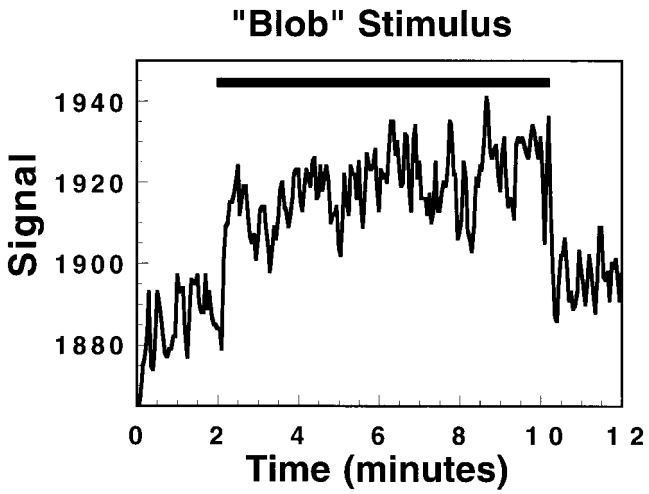

Figure 11.

Blood oxygenation-sensitive (TE = 40 ms, TR = 4 sec, θ = 90°) signal from the visual cortex during 8 min of ''blob'' stimulation. Even though the stimulus selectively activates areas of high mitochondria concentration, the extended duration signal behavior remains unaffected. One small difference in the signal behavior is that an undershoot is not apparent following cessation of the stimulus.

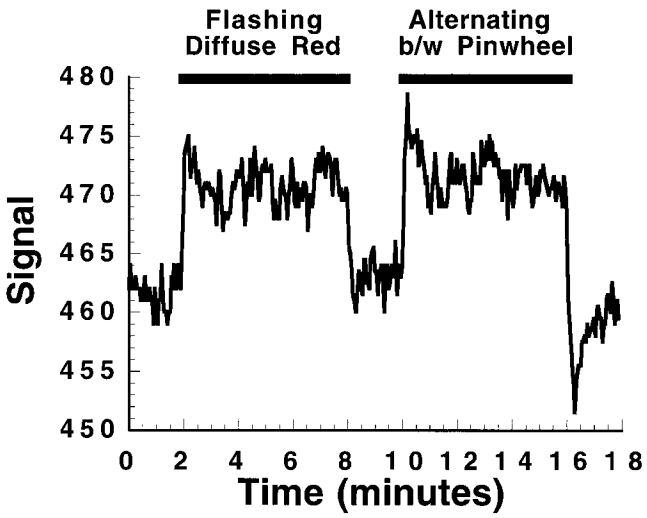

Figure 12.
Blood oxygenation-sensitive (TE = 40 ms, TR = 3 sec, θ = 90°) signal from the visual cortex during blob stimulation (8 Hz flashing diffuse red) and interblob stimulation (8 Hz alternating high spatial frequency black and white pinwheel). The signal remains elevated for the entire 6 min stimulation durations. One notable difference is the presence of a undershoot in the MR signal following the interblob stimulation and the lack of an undershoot following the blob stimulation.

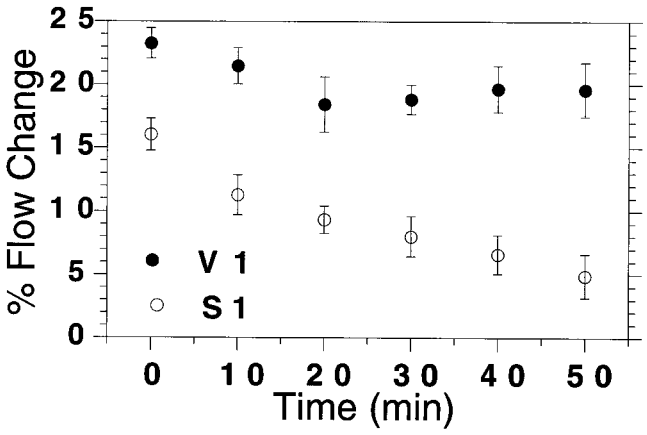

Figure 13.
Flow change characteristics, measured in V1 and S1 in 16 subjects using PET. Flow changes in V1 and S1 were obtained using ^{15}O-labeled water injections at 10 min intervals during 50 min continuous visual and somatosensory stimulation, respectively. A significant flow decrease is observed in S1 but not in V1, demonstrating that extended duration flow responses can vary, due to habituation effects, depending on the stimuli used and cortical regions activated.

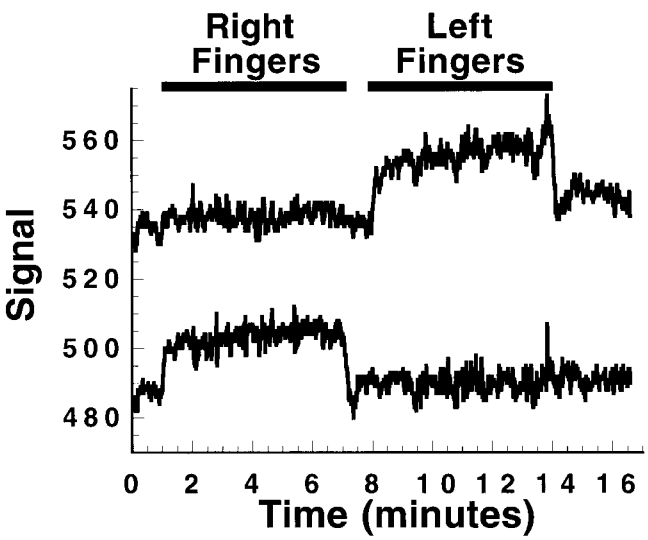

Figure 14.
Blood oxygenation-sensitive (TE = 40 ms, TR = 1 sec, θ = 90°) signal from the left and right motor cortex during 6 min of continuous finger movement. The signal remains elevated, and an undershoot is observed following cessation of movement in each hand. The motor cortex is less likely to demonstrate habituation effects than the sensory cortex.

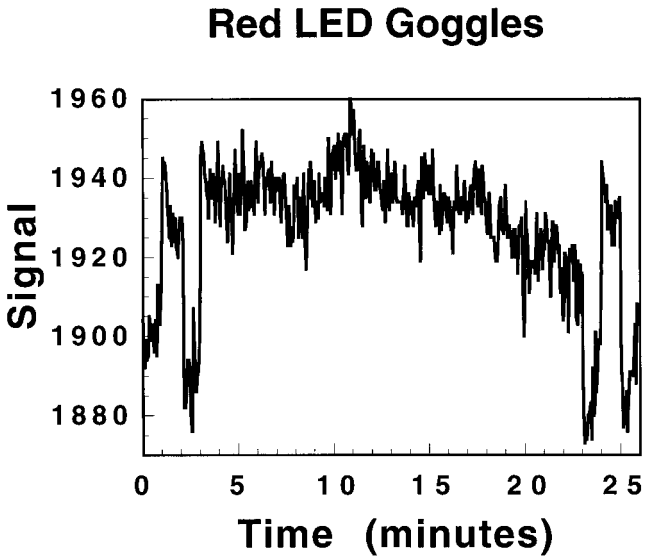

Figure 15.
Blood oxygenation-sensitive (TE = 40 ms, TR = 3 sec, θ = 90°) signal from the visual cortex during 8 Hz flashing diffuse red LED stimuli using Grass goggles. The signal appears to decrease slightly over time, suggesting neuronal habituation.

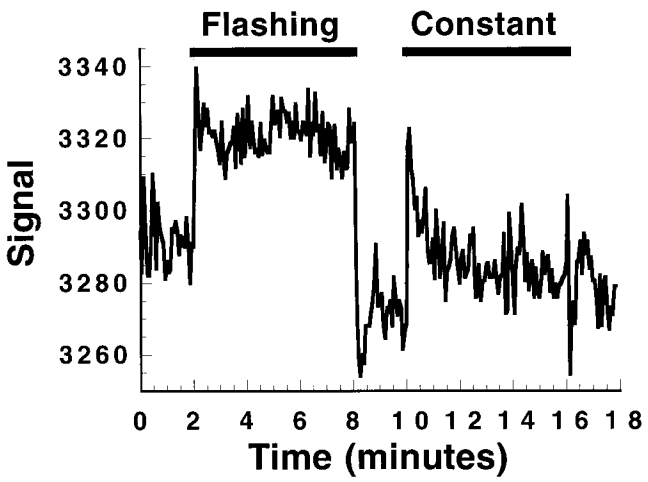

Figure 16.
Blood oxygenation-sensitive (TE = 40 ms, TR = 4 sec, $\theta = 90°$) signal from the visual cortex during either 10 Hz flashing white and during non-flashing white light stimulation. The signal remained elevated during the entire 6 min flashing white light stimulation duration. During the non-flashing white light stimulation, the signal returned to baseline within about 1 min after stimulation onset. The control condition was darkness. This transient effect is a clear demonstration of neuronal habituation.

Summary of results

Table I summarizes the essential results of the paper. As can be seen, the only stimulus that resulted in a consistent decrease in signal after 1 min was the 0 Hz (constant) solid white stimulus. This decrease in the BOLD signal is hypothesized to be caused by decreased neuronal firing (i.e., neuronal habituation) and not by an uncoupling of flow with oxygen consumption. All other stimuli demonstrated sustained oxygenation and flow for at least 7 min following the onset of stimulation. It is important to note that in the study involving PICORE, in which flow and BOLD effects were measured simultaneously, the clear decrease, after about 7 min of stimulation, that was observed in the BOLD signal was matched by a corresponding decrease in flow signal.

DISCUSSION

Part 1: MRI parameter modulation

The data consistently demonstrated that both blood oxygenation and flow (regardless of the sequence used), remained constant and fully elevated for at least 7 min of continuous stimulation. It is hypothesized that the decrease in signal occasionally observed in both oxygenation and flow-weighted sequences after about 7–10 min of continuous stimulation is due to a small amount of neuronal habituation (i.e., decreased neuronal firing causing decreased flow and venous oxygenation), since both BOLD and flow signal decreased in relatively similar amounts and at the same time.

Part 2: neuronal and physiologic parameter modulation

The fact that the BOLD signal remained elevated for both blob and interblob stimuli suggests that baseline and/or activation-related differences in metabolic rate do not have a strong effect on the degree of MR signal change or its extended duration stimuli behavior.

Also, the facts that blob and interblob stimuli gave similar steady-state BOLD signal increase behavior but that the interblob stimulation showed a larger post-stimulation undershoot and a larger initial signal increase before reaching a steady state suggest a difference, at the beginning and cessation of activation, in the time for the metabolic rate to increase and decrease, respectively, relative to flow changes. It may also suggest a stimulus-related difference in blood volume change dynamics relative to flow and oxygenation changes [Buxton et al., 1997], or it may be a result of luminance differences in the stimuli.

It is clear from the PET studies and the luminance change fMRI studies that the degree of neuronal habituation, indicated by flow decreases over time, can vary widely depending on the type of stimulus used. This type of effect should be kept in mind when interpreting extended duration stimulation fMRI results.

The results clearly suggest that the factors causing a decrease (during stimulation) in BOLD-weighted signal are involved with neuronal habituation (i.e., decreased neuronal firing) and not changes in coupling, on the spatial scale at which the observations are made, of oxygenation consumption with oxygenation delivery.

Two other preliminary studies [Howseman et al., 1996; Kollias et al., 1996] have demonstrated results similar to those presented in this paper. These results differ from those of Frahm et al. [1996] as well as from those of Hathout et al. [1994]. Several of our experimental results resembled those of Hathout et al. [1994], suggesting the possibility that their observations might have been due to neuronal habituation effects.

More perplexing are the differences between the results presented and those of Frahm et al. [1996], which show a decrease in BOLD signal almost to

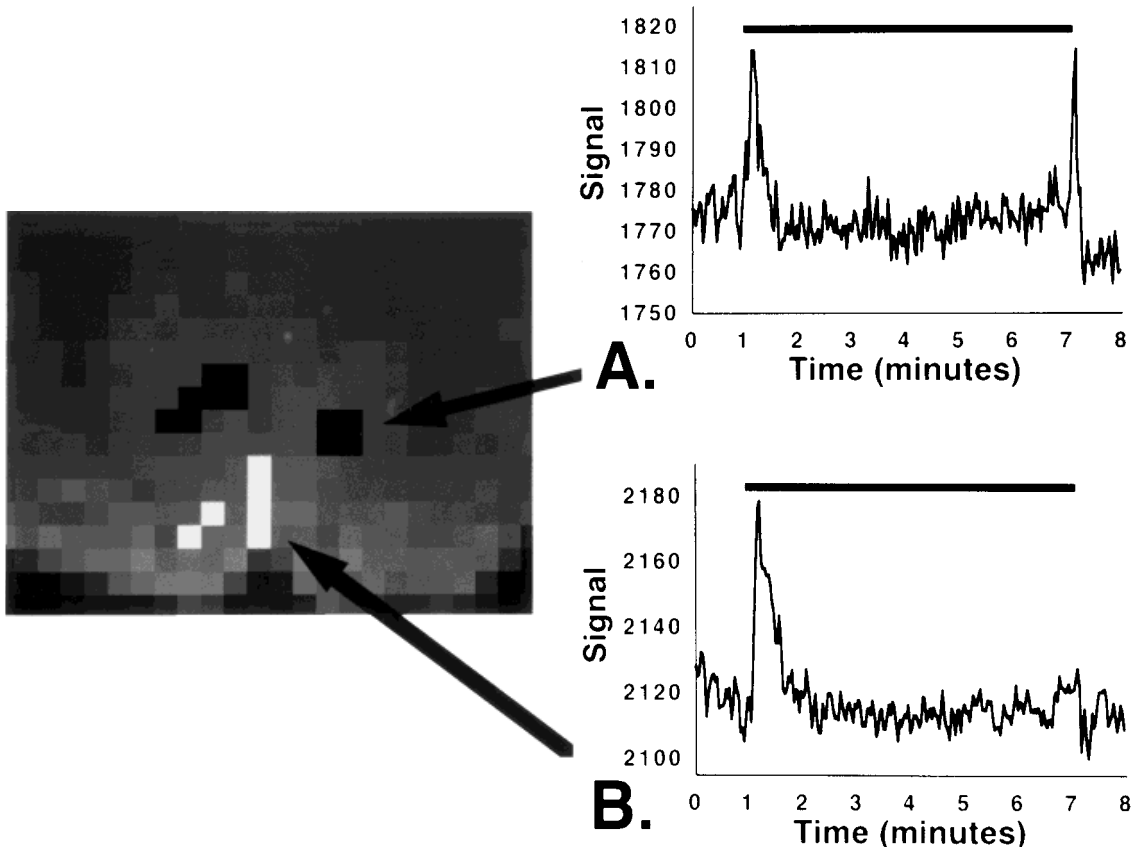

Figure 17.

Blood oxygenation-sensitive (TE = 40 ms, TR = 2 sec, $\theta = 90°$) signal from the visual cortex during steady (non-flashing) white light. The time course of the signal from region **A** (black voxels) and region **B** (white voxels) is shown. The horizontal bars indicate the time at which the stimulus was on. The signal returns to baseline after less than 1 min of the stimulation. The signal from region **A** shows a short (10 sec) burst of activity during the transition from an on to an off state. Signal from region **B** shows less of a signal change during this transition from brightness to darkness.

baseline level after 2 min and then a subsequent undershoot after extended stimulation lasting at least 2 min. Possible explanations may be systematic differences in subject population characteristics, (yet unknown) differences in pulse sequence sensitivities between FLASH and EPI, differences in the stimuli, or differences in the resting state conditions.

A simple biomechanical model proposed by Buxton et al. [1997] is able to reproduce the results of Frahm et al. by allowing that inflow and outflow not necessarily equal each other at all points in time. In other words, the model can predict the results of Frahm et al. and the results presented here by varying the relative time constant of blood volume changes, blood flow changes, and blood oxygenation changes during the onset of activation and the return to baseline. Furthermore, these transient effects can be consistent with a continuous tight coupling between flow and oxidative metabolism [Buxton and Frank, 1997].

Other studies using PET have determined that the oxidative metabolic rate during neuronal activation increases by 5% and either stays constant or increases over time [Marrett, personal communication, 1996]. Studies that seem to agree with the results presented in this paper were recently published [Madsen et al., 1995]. Using the Kety-Schmidt technique, arterial-venous differences in oxygenation were shown to remain steadily decreased during an activation task involving larger cortical regions (Wisconsin card sort task) [Madsen et al., 1995].

The studies presented do not rule out the possibility that an increase in oxidative metabolic rate does not occur during prolonged stimulation or on a smaller spatial scale. The results of Grinvald et al. [1991], Frostig et al. [1990], and Malonek and Grinvald [1996] using optical imaging techniques, as well as Menon et al. [1995a] using fMRI, suggest that small and transient flow / oxygenation coupling changes are detectable. In

TABLE I. Summary of the experimental results

Stimulus	No.	Contrast	Method	Stimulus duration (min)	Signal behavior
10 Hz alternating checkboard	4	BOLD	GE-EPI	20	0–10% attenuation starting after 7 min
	4	Flow	GE-EPI	20	0–10% attenuation starting after 7 min
	1	BOLD	PICORE	20	0–10% attenuation starting after 7 min
	1	Flow	PICORE	20	0–10% attenuation starting after 7 min
	1	BOLD	GE-spiral scan	6	0% attenuation
	1	BOLD	Long TR GE-EPI	20	0% attenuation
8 Hz alternating checkerboard	9	Flow	O-15 PET	50	0–10% attenuation by 50 min
8 Hz vibrotactile	7	Flow	O-15 PET	50	50% attenuation by 20 min 75% attenuation by 50 min
8 Hz "blob" (low spatial frequency color)	2	BOLD	GE-EPI	8	0% attenuation
8 Hz "interblob" (high spatial frequency b/w)	2	BOLD	GE-EPI	8	0% attenuation
8 Hz flashing red LED goggles	2	BOLD	GE-EPI	20	20–50% attenuation starting after 10 min
8 Hz flashing solid white	2	BOLD	GE-EPI	6	0% attenuation
0 Hz (constant) solid white	2	BOLD	GE-EPI	6	100% attenuation by 1 min
5 Hz finger opposition	1	BOLD	GE-EPI	6	0% attenuation
	1	BOLD	ΔTE GE-EPI	4	0% attenuation
	1	Flow	ΔTE GE-EPI	4	0% attenuation

the BOLD signal (reflecting flow, volume, and oxygen extraction fraction changes), it is conceivable that a small change in oxidative metabolic rate over time, and during an already elevated flow state, may not cause a change in venous oxygenation detectable by fMRI. Further study is necessary to characterize these effects more fully.

CONCLUSIONS

An array of studies has demonstrated that both flow and oxygenation remained elevated for the entire stimulation durations, except when stimuli known to cause neuronal habituation (decreased neuronal firing) were used. Pulse sequence strategies allowing exclusive flow and oxygenation sensitivity were used to confirm that flow and oxygenation remain elevated during prolonged activation. Also, multi-shot minimally flow sensitive T2*-weighted spiral scanning was performed during extended duration stimulation. Oxygenation remained elevated during the entire stimulus duration.

Stimuli known to activate tissue having different mitochondria concentrations elicited no differences in extended duration behavior of blood oxygenation, although a post-stimulation undershoot was more apparent following the stimulation involving black and white alternating high spatial frequency (interblob) stimulus than following the diffuse red (blob) stimulus.

The effects of extended stimulation known to have different neuronal habituation effects were tested. Neuronal habituation effects were observed in V1 after about 7 min using 8 Hz flashing red LED goggles. Clear neuronal habituation effects were demonstrated in V1 using a featureless and steady-state white visual

stimulation. With this stimulus, the BOLD signal returned to baseline after about 1 min. Some regions showed a brief signal increase on returning to the off state.

These studies strongly suggest that flow and oxygenation, as measured on this spatial scale (approximately 3 mm^3) remain elevated and constant for the entire duration that neurons are firing at a constant rate. The observed decreases in both oxygenation and flow over time are attributed to neuronal habituation, which corresponds to a decrease in neuronal firing rate with specific types of stimuli.

Future efforts at elucidation of these changes include more precise hemodynamic sensitization (i.e., capillary perfusion, venous perfusion, blood volume) by MRI, higher resolution MRI techniques, more carefully matched experiments across imaging platforms and laboratories, and more careful and complete dynamic modeling of the MRI signal changes such as those proposed by Hathout et al. [1995], Davis et al. [1994], and Buxton et al. [1997].

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